OceanDoctor BWMS

Summary of land based test

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Foreword

According to the IMO BWM Convention and the Guidelines (G8), the land-based testing serves to determine the biological efficacy and environmental acceptability of the BWMS under consideration for Type Approval. The approval testing aims to ensure replicability and comparability to other treatment equipment.

To this end, ten test cycles were conducted by Jiujiang Precision Measuring Technology Research Institute, in accordance with the IMO Convention and the Procedures (G9) and Guidelines (G8). Biological efficacy testing was performed in each of these ten test cycles. Meanwhile, two test cycles were chosen for ecotoxicity test and chemical analysis to the treated water discharge in conditions of max UV dose to evaluate the environmental acceptability of the discharged water treated by OceanDoctor BWMS .The test results and data acquired will be documented as the application dossier to be submitted to the MEPC organized by IMO.

1Test base

The test will be conducted in Ballast Water Detecting Laboratory of Shanghai Ocean University Land-Based Test Base. This test base is located at the No.2 dockin the Xiaoyang Hill in Shanghai, in East China Sea. The photo of the test base is shown in the figure 1.1:

Figure 1.1 picture of the test base

2 Test set-up

The test set-up is mainly composed of a OceanDoctor BWMS with a TRC of 250 m^3/h and the purpose-built test facility which is provided by Ballast Water Detecting Lab of Shanghai Ocean University Land-based Test Base. This test set-up can realize the function of water ballasting, storage and

discharge to meet the test requirements of G8.

2.1 the test equipment

The equipment was tested is a full scale ballast water management system with a TRC of 250m^3/h. It is mainly encompassed by the filtration unit, the photo-catalytic reaction unit, the control unit. The system is applied to disinfect the aquatic organisms like algae and bacteria in ballast water. The drawing of the BWMS is as shown in figure 2.1.

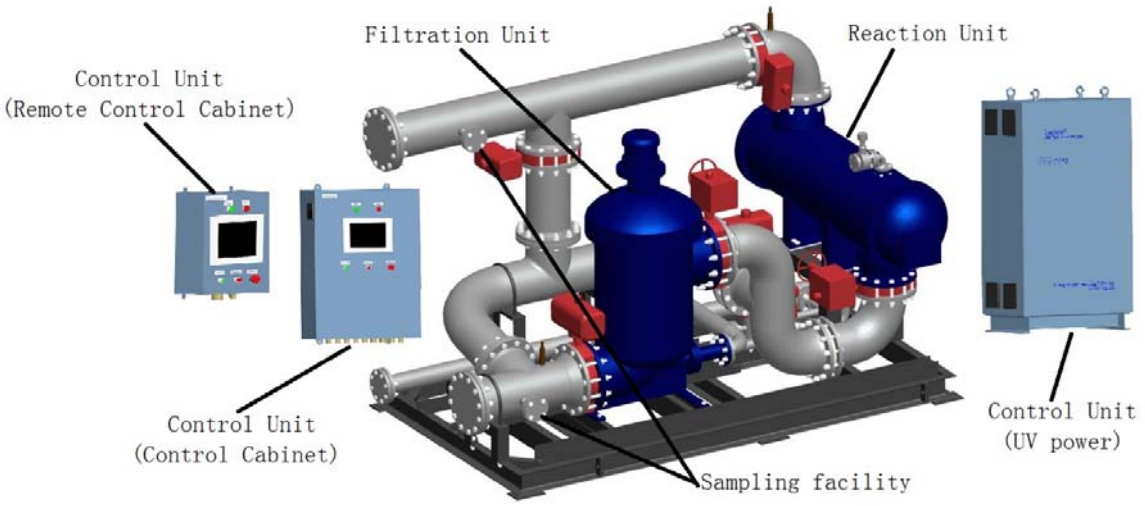


Figure 2.1 test equipment

2.2 test faciltiy

The test facility is mainly composed of a ballast pump, a treated tank, a control tank, a water feed tank, a fresh water tank, sampling facility and so on. The arrangement and the features of the test facility comply with the requirements of G8. The configuration of the test facility is shown in figure 2.2:

Ballast pump: it will be utilized to pump the water to the ballast water management system. The capacity of ballast pump is 300 m^3/h.

Treated tank: it will be utilized to store the treated ballast water with a volume

of 250m^3. The inside wall of the tank is covered with marine cabin paints.

Control tank: it will be used to store the untreated ballast water with a volume of 250m^3. The inside wall of the tank is covered with marine cabin paints.

Feed tank: an aeration device is installed inside of it. This tank is utilized as a container for intake water augementation to achieve challenge conditions. The volume of the feed tank is 500 m^3.

Fresh water tank: it will be used for fresh water storage to wash the test system and pipe prior to or after test. The volume of it is 50m^3.

Sampling facility: there are two sampling facilities equipped in the test set-up and made up of seven sampling points (S3, S4, S5, S6, S7 is the same point with S1, which are utilized to take different samples), S1 is used for influent water sampling; S2 is used for treated water sampling; S3 is utilized for control water sampling taking from the control tank; S4 is used to take treated water samples after holding for 24h; S5 used to take treated water samples after holding for 120h; S6 is used to take control water samples after holding for 120h; S7 is used to take control water samples after a 24h hold.

The design of the sampling facility strictly follows the technical specifications of sampling facilities introduced in G2. The structure of the sampling facility is shown in figure 2.3:

Piping: direct the sea water in the pipe by adjusting the valve.

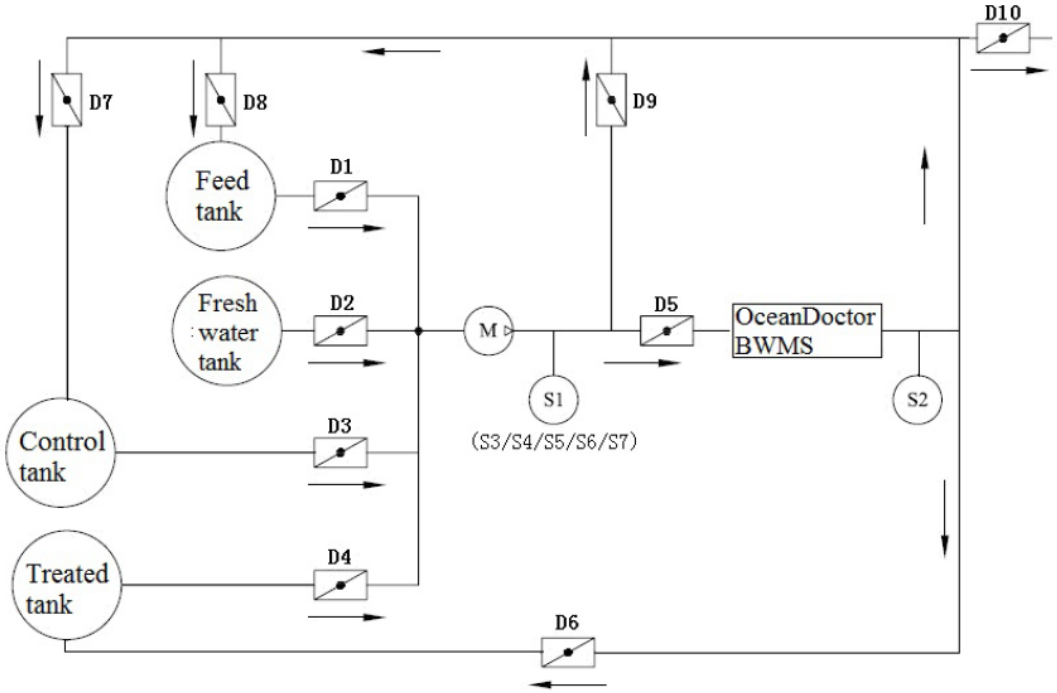


Figure 2.2 diagram drawing of the test set up

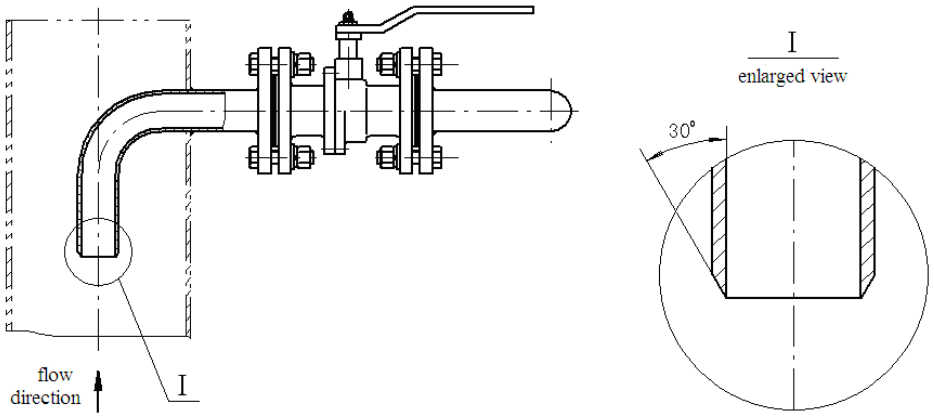


Figure 2.3 structure of the sampling facility

3 Test water

3.1 Regulations on test water

3.1.1 regulations on water quality

At least two sets of tests cycles (5 replicates is considered a set)should be conducted, each with a different regime of salinity and associated DOC (dissolved organic carbon), POC (particulate organic carbon) and TSS (total suspended solids) content. Tests under adjacent salinity ranges in the

following table should be separated by at least 10 PSU. 10 test cycles are carried out by us, 5 test cycles at the regime of >32 PSU and the other at the regime of 3-32 PSU.

According to G8, the water quality and the salinity range of the challenge water should meet the requirements as indicated in table 3.1:

Table 3.1 requirement for influent water

	salinity	(DOC） Dissolved organic carbon	(POC） Particulate organic carbon	(TSS） Total suspend solid	test cycles
1	3-32PSU	>5mg/l	>5mg/l	>50mg/l	5
2	>32 PSU	>1mg/l	>1mg/l	>1mg/l	5

3.1.2 regulations on micro organisms

Test organisms may be either naturally occurring in the test water, or cultured species that may be added to the test water. If cultured test organisms are used, it should be ensured that local applicable quarantine regulations are taken into account during culturing and discharge. The organism concentration should comply with paragraph below:

(1) test organisms of greater than or equal to 50 micrometers or bigger in minimum dimension should be present in a total density of preferably 10_6 but not less than 10^5 individuals per cubic meter, and should consist of at least 5 species from at least 3 different phyla/divisions;

(2) test organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension should be present in a total density of preferably 10^4 but not less than 10^3 individuals per milliliter, and should consist of at least 5 species from at least 3 different phyla/divisions;

(3) heterotrophic bacteria should be present in a density of at least 10^4 living bacteria per milliliter; and

(4) the variety of organisms in the test water should be documented according to the size classes mentioned above regardless if natural organism assemblages or cultured organisms were used to meet the density and organism variety requirements.

(5) the following bacteria do not need to be added to the influent water, but should be measured at the influent and at the time of discharge:

① Coliform;

② Enterococcus group;

③ *Vibrio cholerae*; and

④ Heterotrophic bacteria.

3.2 Preparation of the test water

Based on the requirements on test water, the test cycles conducted for land-based test include the high salinity test cycles（>32 PSU）and the medium salinity test cycles（3-32 PSU）.

(1)Preparation of high salinity seawater

High salinity test cycles: natural seawater is taken from the east sea area of Sheng shan island of Zhoushan city (S_5 in figure5.4) which is 50 nautical miles from the test base, then pumped to the feed tank. Because the salinity of the natural seawater is about 32.2~33.0 PSU, it was utilized as the high salinity test water directly.The specific salinity of the seawater was measured.

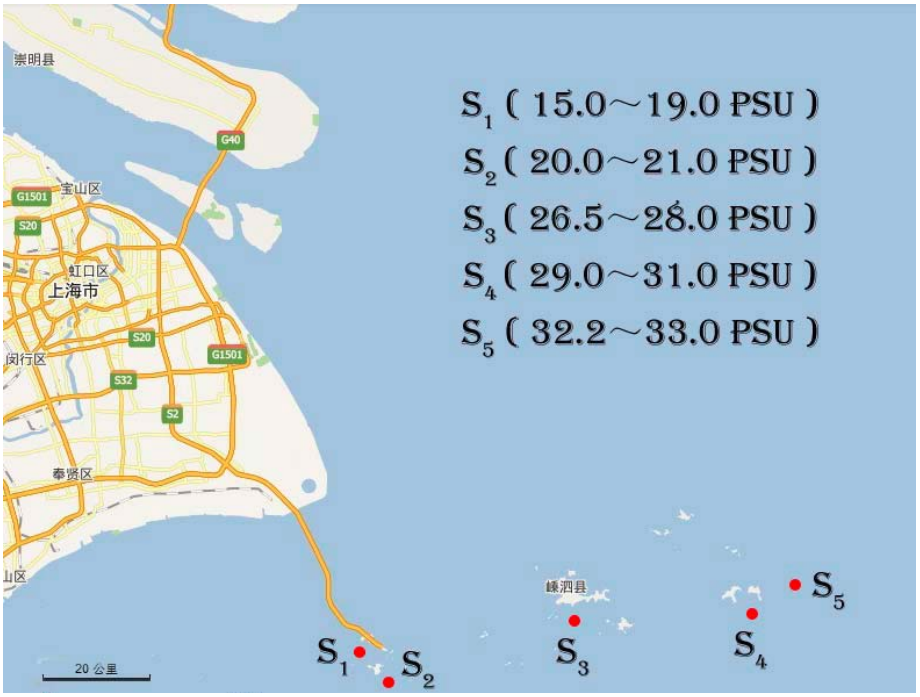


Figure 3.1 Map of the Location of High Salinity Test Water

(2) Preparation of medium salinity seawater

Medium salinity test cycles: Natural seawater in the test base was taken directly as the medium salinity seawater (S_1 in figure 3.1). The salinity of the natural seawater in the test base is about 19.0 PSU; the specific salinity of the seawater was measured. The salinity of the high salinity water is about 33 PSU; the two salinities are separated by about 14PSU, which meets the requirement of G8.

(3)Preparation of organisms

The marine organisms was cultured by Shanghai Ocean University and added to the challenge water as needed. The cultured microorganisms at a specific density were injected into the feed tank and an air pump was utilized in combination with the aeration device to inflate the water. On one hand, this action could keep the activeness of the organism; on the other hand, it made the organisms be evenly spreading in water.

(4) Preparation of humic acid and soil

When test water of different salinity cannot meet specific requirements in

terms of DOC, POC and TSS, certain amount (according to actual measurement) of humic acid and soil must be added. Scatter the additives evenly and then fully stir the water with the air pump.

4 Test cycles

4.1 Test cycle steps

Two sets of test cycles (5 replicates is considered a set) were conducted .five test cycles were conducted with a high salinity (>32PSU), and the other five test cycles were conducted with a medium salinity (3-32 PSU). Biological efficacy testing was performed in each of these ten test cycles,.meanwhile, two test cycles named test cycle 3 and test cycle 4) were chosen for ecotoxicity test and chemical analysis to the treated water discharge in conditions of max UV dose. The four high salinity test cycles and four medium salinity test cycles were conducted under condition of minimum UV dose, and water samples for biological efficacy test were collected from the influent, the treated water.

A complete test cycle consists of three phases: ballasting, holding and deballasting. Water was sampled during ballasting and on discharge. The samples collected were pretreated and delivered to the test organizations in accordance with associated transportation and storage requirements to ensure the validity of the test results.

4.1.1 Ballasting

On the 0 day of test: the augmented water in the feed tank was pumped to the test equipment. The ballast water flew through the filtration unit, and then the photocatalytic reaction unit and finally to the treated tank. And the untreated ballst track flows to the control tank.

4.1.2 storage

① the treated ballast water was held in the treated tank for 120h. The untreated ballast water was stored in the control retention tank for 120h alike.

②the treated tank and the contol tank was ventilated while lightproof.

4.1.3 Deballasting

On the 5 day of the test: the treated water in treated tank and the control water in the control tank was discharged.

4.2 Preventing cross-contamination between cycles

To avoid cross contamination between different test cycles, the following measures were taken to control the test process.

⑴During the test cycle phase, each test step and test cycle, connect the test pipes to the fresh water tank and switch on the ballast pump and management system to flush the in-line pipes. The amount of fresh water is three times larger than the total capacity of the BWMS and test pipes. Discharge all the fresh water after washing.

⑵After each test cycle is over, drain the feed tank, the treated tank and the control tank. And then, cleanse the treated tank with freshwater, then drain the freshwater, by utilizing drier to dry the feed tank, the treated tank and the control tank.

⑶Before the test cycle, sampling equipment and containers used in test cycles need to be pretreated according to requirements of test organizations. If the sampling equipment and containers need to be recycled, they should be treated after usage to avoid affecting the following tests.

5 test contents

According to the requirements of the land-based test, the testing category is detailed as follows:

(1) Biological efficacy test;

(2) Chemical analysis;

(3) Ecotoxicity test;

5.1 Biological efficacy test

5.1.1 Test standard

The biological efficacy of the BWMS is evaluated in accordance with requirements of land-based testing designated in Guidelines for Approval of Ballast Water Management System (G8). Test water must be prepared according to the requirements on influent water quality and organism concentration in land-based testing. After treatment, the microbe and bacteria concentration in the discharged water should be in compliance with the values in regulation of D-2: Regulation D-2 stipulates that ships meeting the requirements of the Convention by meeting the ballast water performance standard must discharge:

(1) Discharge less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension, and

(2) Less than 10 viable organisms per millilitre less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and

(3) Indicator microbes, as a human health standard, shall include:

① Toxicogenic Vibrio cholerae (O1 and O139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gram (wet weight) zooplankton samples;

② Escherichia coli less than 250 cfu per 100 milliliters;

③ Enterococci less than 100 cfu per 100 milliliters.

5.1.2 Test items

(1) Test items for biological efficacy:

① Enumeration of test organisms of greater than or equal to 50 micrometers or greater in minimum dimension

② Enumeration of test organisms of greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension

③Total count of the Heterotrophic bacteria

④ Total count of Coliform

⑤ Total count of Enterococcus group

⑥ Total count of Vibrio cholerae

(2) Environmental Parameters

① pH

② Temperature

③ Salinity

④ Dissolved oxygen (DO)

⑤ Total Suspended Solids (TSS)

⑥ Dissolved Organic Carbon (DOC)

⑦ Particulate Organic Carbon (POC)

⑧Turbidity (NTU)

⑨ Total residual oxident (TRO)

5.1.3 Test facility/organization

Ballast Water Detecting Lab of Shanghai Ocean University was entrusted to conduct the biological efficacy test and evaluate the treatment efficiency of the ballast water management system.

5.2 Chemical analysis

5.2.1 Test items

According to Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9), active substances, preparations used by BWMS and relevant chemicals that produced in the process of treatment should be evaluated to determine their effects on aquatic organisms and environment.

5.2.1.1 Relevant chemicals

Through analiyzing the technical characteristic of our system and also consulting relevant information and literatures, a testing list for chemicals analyzed is given in the following table:

Table 5.1 test list for chemicals

No.	Name	*CAS No.*	No.	Name	*CAS No.*
1	Tribromomethane	*75-25-2*	26	Dichloroacetonitrile	*3018-12-*

					0
2	Trichloromethane	*67-66-3*	27	Trichloroacetonitrile	*545-06-2*
3	Dibromochloromethane	*124-48-1*	28	Chloralic hydrate	*75-87-6*
4	Bromodichloromethane	*75-27-4*	29	2,4- Dibromophenol	*615-58-7*
5	Bromochloromethane	*74-97-5*	30	2,6- Dibromophenol	*608-33-3*
6	Dibromomethane	*74-95-3*	31	2,4,6-Tribromophenol	*118-79-6*
7	Dichloromethane	*75-09-2*	32	2- chlorophenol	*95-57-8*
8	1,2-Dibromo-3-Chloropropane	*96-12-8*	33	3- chlorophenol	*108-43-0*
9	1,2- Dichloropropane	*78-87-5*	34	4- chlorophenol	*106-48-9*
10	1,2,3-Trichloropropane	*96-18-4*	35	2,3-Dichlorophenol	*576-24-9*
11	2- Chlorotoluene（C7H7Cl）	*95-49-8*	36	2,6-Dichlorophenol	*87-65-0*
12	4- Chlorotoluene（C7H7Cl）	*106-43-4*	37	3,4- Dichlorophenol	*95-77-2*
13	1,2,4- Tribromobenzene（C6h3br3）	*615-54-3*	38	3,5- Dichlorophenol	*591-35-5*
14	Bromoacetic Acid	*79-08-3*	39	2,3,4-Trichlorophenol	*15950-66-0*
15	Dibromoacetic Acid	*631-64-1*	40	2,3,5-Trichlorophenol	*933-78-8*
16	Tribromoacetic Acid	*75-96-7*	41	2,3,6-Trichlorophenol	*933-75-5*
17	Chloroacetic Acid	*79-11-8*	42	2,4,6-Trichlorophenol	*88-06-2*
18	Dichloroacetic Acid	*79-43-6*	43	1,1,1-Trichloroethane	*5471-55-6*
19	Trichloroacetic Acid	*76-03-9*	44	2,3,4,5-Tetrachlorophenol	*4901-51-3*
20	Bromochloroacetic Acid	*5589-96-8*	45	2,3,4,6-Tetrachlorophenol	*58-90-2*

21	Bromodichloroacetic Acid	*71133-14-7*	46	2,3,5,6-Tetrachlorophenol	*935-95-5*
22	Chlorodibromoacetic Acid	*5278-95-5*	47	Pentachlorophenol	*87-86-5*
23	Bromoacetonitrile	*3252-43-5*	48	Carbon tetrachloride	*56-23-5*
24	Dibromoacetonitrile	*3252-43-5*	49	Carbon dichloride	*127-18-4*
25	Bromochloroacetonitrile	*83463-62-1*	50	Bromate	*NA*

5.2.2 Test organization

Shanghai Pony Test Technical Co., Ltd (short for PONY Test) will be entrusted to conduct the test to water samples collected over the course of the test for analyzing the relevant chemicals.

5.3 Ecotoxicity Test

According to Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9), ballast water treated with Active Substances should be subject to ecotoxicity test to identify its harmful effects on environment.

5.3.1 Test items

(1) Acute toxicity test

① Algal growth inhibition test

② Fish acute toxicity test

③ Crustacea acute toxicity test

(2) Chronic toxicity test

① Fish chronic toxicity test

② Crustacea chronic toxicity test

5.3.2 Test organization

Supervision and Test Center for Pesticide Safety Evaluation and Quality Control conducted the acute toxicity and chronic toxicity tests for treated ballast water.

5.4 Test schedule

There were 10 test cycles performed in the land-based test, which including five high salinity test cycles(#4, #5, #6, #7, #8) and five medium salinity test cycles(#1, #2, #3, #9, #10). Samples were taken at each of the ten test cycles for biological efficacy test. Meanwhile, samples taken at test cycle #3 and #4 under conditions of 150 m^3/h of the flow rate（max UV dose）were used for chemical analysis and ecotoxicity test. Samples taken at test cycle #8 and #9 under conditions of 275 m^3/h of the flow rate（min UV dose）were used for biological efficacy test. The other six test cycles are conducted under the condition of rated flow rate（average UV dose）, during which, water was sampled for biological efficacy test.

The test process is as listed in the following table (the time for formal test cycle is referred as the 0h, and hereinafter inclusive)

Table 5.2 process of land-based test

Test cycle	Date	Work carried out
Test cycle #1 （medium salinity, 250 m3/h）	2012.0802-2012.0807	Test was started, regulate the flow to 250 m3/h; ballast water was treated in the BWMS. Biological efficacy samples were collected, after being pretreated, the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #2 （medium salinity, 250 m3/h）	2012.0809-2012.0814	Test was started, ballast water was treated in the BWMS, biological efficacywater samples were collected.After being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #3 （medium	2012.0816-2012.0821	Test was started, regulate the flow to 150 m3/h, ballast water was treated in the BWMS, water was sampled for testing of

salinity, 150 m3/h）		biological efficacy, relevant chemicals, ecotoxicity.After being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #4 （high salinity, 150 m3/h）	2012.0823-2012.0828	Test was started, ballast water was treated in the BWMS, and water was sampled for testing of biological efficacy, relevant chemicals, ecotoxicity. After being pretreated, the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #5 （high salinity, 250 m3/h）	2012.0830-2012.0904	Test was started, regulate the flow to 250 m3/h, ballast water was treated in the BWMS, biological efficacy water samples were collected.After being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #6 （high salinity, 250 m3/h）	2012.0906-2012.0911	Test was started, ballast water was treated in the BWMS, biological efficacywater samples were collected.After being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #7 （high salinity, 250 m3/h）	2012.0913-2012.0918	Test was started, ballast water was treated in the BWMS, biological efficacysamples were taken.after being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #8 （high salinity, 275 m3/h）	2012.0920-2012.0925	Test was started, regulate the flow to 275 m3/h, ballast water was treated in the BWMS, biological efficacy water samples were collected.After being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle	2012.0927-2012.1002	Test was started, ballast water was treated in

#9（medium salinity, 275 m3/h）		the BWMS, biological efficacywater samples were collected.After being pretreated, and the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.
test cycle #10（medium salinity, 250 m3/h）	2012.1004-2012.1009	Test was started, regulate the flow to 250 m3/h, ballast water was treated in the BWMS, and biological efficacywater samples were collected. After being pretreated, the samples were delivered to the test organization. The test organization carried out the testing to the samples and submitted the test report.

6 sampling

6.1 Sampling protocal

The sampling protocol should result in samples that are representative and in random with appropriate sample size, and the sampling method should be in line with these G2 Guidelines:

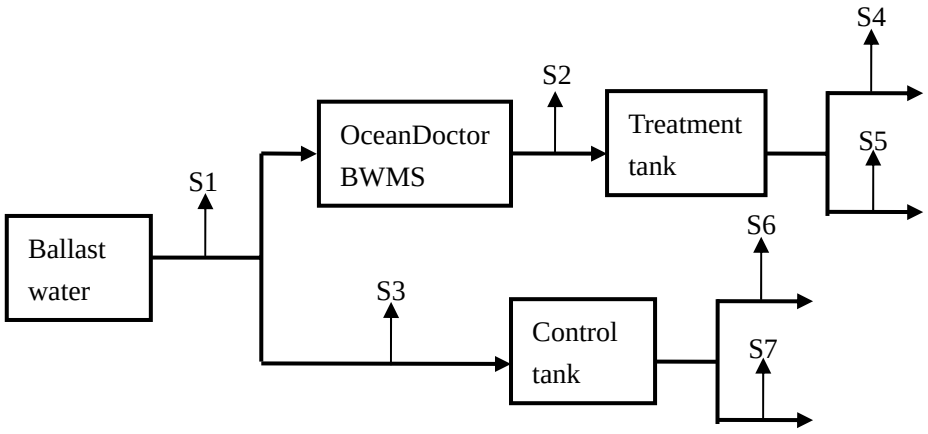
Sampling should meet the requirements in G2 which could be summarized as follows: The samples should be representative of the whole discharged ballast water; the sampling protocol should take account of the potential for a suspended substance in the discharge to affect sample results; the quantity and quality of samples taken should be sufficient to demonstrate whether the quality of discharged ballast water meets the D-2 discharge standard; sampling should be undertaken in a safe and practical manner; samples should be concentrated to a manageable size; samples should be taken, sealed and stored to ensure that they can be used to test in accordance with the Convention, and the whole process of sampling, transport, handling and storage should be documented; samples should be fully analyzed following the test method holding time limit by an accredited laboratory.

According to G2 and G8 Guidelines and IMO Convention as well as

requirements of test Organizations, samples for biological efficacy, relevant chemicals and ecotoxicity tests are collected respectively and transported to relevant laboratories in accordance with storage and transport regulations.

6.2 sampling point

Ten test cycles were conducted including 5 high salinity test cycles and 5 low salinity test cycles. All ballasting, holding and deballasting operations were realized by the test set-up. Two sampling facilities are equipped in the test set-up and made up of seven sampling points used to collect different water samples for testing of biological efficacy, relevant chemicals and ecotoxicity. As shown in figure 6.1 and table 6.1.



S1: influent water S2: treated water S3: control water

S4: treated water after a 24h hold S5: treated water after a120h hold

S6: control water after a 120h hold S7: control water after a 24h hold

Figure 6.1 diagram of the sampling points' arrangement

Table 6.1 information on the collected samples

sample	**sampling point**	**sample volume**	**remarks**
influent	S1	20L×3	≥50μm

water		1L×3	10-50μm
		0.7L×3	bacteria
		5L×3	water quality
		14 L × 2	chemical analysis
		> 700 L	ecotoxicity
treated water	S2	$1m^3$×3	≥50μm
		10 L×3	10-50μm
		0.7L×3	bacteria
		5L×3	water quality
		14 L × 2	chemical analysis
		> 500 L	ecotoxicity
control water	S3	20L×3	≥50μm
		1L×3	10-50μm
		0.7L×3	bacteria
		5L×3	water quality
treated water after a 24h hold	S4	14 L × 2	chemical analysis
		> 500 L	ecotoxicity
control water after a 24h hold	S7	> 700 L	ecotoxicity
treated water after a 120h hold	S5	$1m^3$×3	≥50μm
		10 L×3	10-50μm
		0.7L×3	bacteria
		5L×3	water quality
		14 L × 2	chemical analysis
control water after a 120h hold	S6	$1m^3$×3	≥50μm
		10 L×3	10-50μm
		0.7L×3	bacteria
		5L×3	water quality

6.3 sampling methods

To take representative and random samples, keep the BWMS running for 10 minutes before sample collection. Then put sample containers under the

sampling points until the container is close to being full; add prepared fixative or stain to the container, shaking it to make the additives mix thoroughly; replenish the container with the same sample water and put it in cold storage after sealing and marking. When conducting replicate sampling, stick to a sequence of beginning, middle, end at a 15 minutes interval.

To ensure the randomness of the samples, replicate samples need to be taken in each of the sampling point (beginning, middle, end). Triplicate samples were taken for biological efficacy test each at the 10min,25min,40min after the start of the test. Two replicate samples were taken for chemical analysis each at 10min and 25min after the start of the test; and one replicate sample was taken for ecotxicity test at 10min after the start of the system. For specific approaches and points for attention, please refer to the QAPP from relevant test Organizations. Sampling record sheet should be filled after finishing sampling.

7 test result

Ten test cycles were conducted in accordance with the requirements of IMO Convention and G8, G9. The test results indicate that the biological efficacy, the ecotocixity and chemical analysis to the ballast water treated by the OceanDoctor BWMS fulfill the requirements of IMO Convention and G8, G9.

7.1biological efficacy test result

What presented here is quoted from land based summary and analysis test report, please refer to the appendix I for detail.

Land based test to OceanDoctor BWMS developed by Jiujiang Precision Measuring Technology Research Institute was conducted at the land based test base of Ballast Water Detecting Lab of Shanghai Ocean University from 2012.8~2012.10. A summary of the results from all phases is included in this document compared with what is required by regulation D-2 and G8:

1. fulfill the chemical requirement of the test water

Average value of water quality tested at the uptake in the 5 medium salinity test cycles are: average water temperature 27.22 ℃,salinity: 16.74 PSU,POC:5.24 mg/L,TSS:54.38 mg/L,DOC: 8.22 mg/L;

Average value of water quality tested at uptake in the 5 medium salinity test cycles are: average water temperature: 27.76℃,salinity: 32.6PSU,POC: 2.39mg/L,TSS: 25.76 mg/L,DOC: 8.06 mg/L. The POC, TSS and DOC in the test water of the ten test cycles fully meet the requirements as stipulated in G8, and the high salinity ranges and medium salinity ranges are separated by more than 10 PSU which meet the requirement of G8 as well.

2. fulfill the biological requirement of the test water

2.1 Organisms ≥50μm

Organisms which are ≥ 50μm in minimum dimension in the test water are composed of both the natural marine organisms and the added organisms. The averagce density of the organism in the influent water in medimum salinity test cycle is 3.25×10^5 ind/m^3, and in high salinity test cycle is 2.47×10^5 ind/m^3, The organims in the test water belong to the arthropoda, algae and rotifera. The main species are listed as Brine Shrimp, Paracalanus aculeatus, Limnoithona tetraspin, Coscinodiscus spp, Brachionus sp and etc, among which the Brine Shrimp is the dominant specie. Therefore, the organisms in the test water meets the requirement of G8. The density of organisms ≥50μm in high salinity test cycles in the treated water after a 120h storage is 1.93 ind/m^3, and the density of organisms≥50μm in the control water after a 120h storage is 2.30×10^4 ind/m^3;

The density of organisms ≥50μm in high salinity test cycles in the treated water after a 120h storage is 1.8 ind/m3, and the density of organisms≥50μm in the control water after a 120h storage is 1.43×10^4 ind/m3;

It indicates that all meet the requirements of G8 and regulation D-2.

2.2. Organisms 10～50μm

Organisms which are 10～50μm in minimum dimension in the test water are composed of both the natural marine organisms and the added organisms.

The algae in the test water belong to the Chlorophyta, Euglenophyta and Bacillariophyta. The main species are listed as Amphora coffeaeformis, Platymonas sp. and so on, and besides of that, there are Trachelomonas sp., Cyclotella spp., Skeletonema costatu, Thalassiosira nordenskioldii, Amphora ovalis and etc, among which the platymonas sp. and the platymonas subcordiformis are the dominant species.

The average concentration of organisms 10～50μm in minimum dimension in the medium salinity test cycles is 2.18×10^3 cells /mL, and in high salinity test cycles is 3.37×10^3 cells/mL, the test water meets the biological requirement of G8. concentration of organism of this size group in medium salinity test cycles is 0 cells/mL in treated water after a 120h storage, while organism concentration in the control discharge is 208.75 cells/m.

The average concentration of organisms 10～50μm in minimum dimension in the high salinity test cycles is 0.26 cells/mL, and in high salinity test cycles is 3.37×10^3 cells/mL, the test water meets the biological requirement of G8. concentration of organism of this size group in medium salinity test cycles is0 cells/mL in treated water after a 120h storage, while organism concentration in the control discharge is 207.28 cells/mL.

It indicates that all meet the requirements of G8 and regulation D-2.

2.3. bacteria

Intestinal Enterococci is added to augment the test water in order to meet the requirement of G8. The density of heterotrophic bacteria in the influent is larger than 5.0×10^4cfu/mL, which meets the requirement of G8. The average density of heterotrophic bacteria in treated water after a 120h storage in medium salinity test cycles is 140.2cfu /mL, and 1.71×10^4cfu/mL in the control water after a 120h storage. The average density of heterotrophic bacteria in treated water after a 120h storage in high salinity test cycles is113.86cfu /mL, and 4.72×10^4cfu/mL in the control water after a 120h storage.

The average density of Intestinal Enterococci in treated water after a 120h storage in medium salinity test cycles is 26.12 cfu /mL, and 4.68×10^3cfu/mL in

the control water after a 120h storage. The average density of Intestinal Enterococci in treated water after a 120h storage in high salinity test cycles is 15cfu /mL, and 4.22×10^3 cfu/mL in the control water after a 120h storage.

It indicates that all meet the requirements of G8 and regulation D-2.

No Enterococcus group and Vibrio cholerae is detected in the treated water in the ten test cycle. Therefore, the test results of bacteria are in compliance with G8 and regulation D-2.

In conclusion, the chemical and biological parameters in the influent and the control discharge in the ten test cycles all meet the requirement of G8. The treated discharge meets the standards as set out in regulation D-2 and G8.

7.2 chemical analysis test result

Documents related to the chemical anaylisis (QAPPs and test report) have been submitted to the IMO and reviewed by the experts from GESAMP, and final approval has been granted to OceanDoctor BWMS.

The potential by-products in the seawater treated by OceanDoctor BWMS have been tested and analyzed by Shanghai Pony Test Technical CO., Ltd. Based on the analysis results, the Active Substances, Reletive Chemicals and Other Chemicals are listed in 7.1.

Table 7.1 List of Active Substances, Relevant Chemicals and Other Chemicals

Chemical	CAS No	Molar mass	Molecular formula	AS/RC/OC
Hydroxyl redical	3325-57-6	17.01	$\cdot OH$	AS
Tribromomethane	75-25-2	252.77	$CHBr_3$	RC
Trichloromethane	67-66-3	119.38	$CHCl_3$	RC
Dibromochloromethane	124-48-1	208.28	$CHBr_2Cl$	RC
Dichlorobromomethane	75-27-4	163.8	$CHBrCl_2$	RC
Dichloromethne	75-09-2	84.93	CH_2Cl_2	RC
Carbon tetrachloride	56-23-5	153.82	CCl_4	RC

Monobromoacetic acid	79-08-3	138.95	$C_2H_3BrO_2$	RC
Dibromoacetic acid	631-64-1	217.84	$C_2H_2Br_2O_2$	RC
Tribromoacetic acid	75-96-7	296.74	$C_2HBr_3O_2$	RC
Monochloroacetic acid	79-11-8	94.50	$C_2H_3ClO_2$	RC
Dichloroacetic acid	79-43-6	128.9	$C_2H_2Cl_2O_2$	RC

Chemical analysis test result is to evaluate whether any adverse environmental effect is to be expected.

Refer to appendix 2 of this document for detail.

7.3 ecotoxicity test result

Documents related to the ecotoxicity test (QAPPs and test report)have been submitted to the IMO and reviewed by the experts from GESAMP, and final approval has been granted to OceanDoctor BWMS.

The residual effects of treated ballast water were analyzed with acute and chronic bioassays using species from three trophic levels, which were performed by Supervision and Test Center for Pesticide Safety Evaluation and Quality control.

In test cycle 3 and 4, both treated water 0h (immediately after treatment) and after 24h were performed for aquatic toxicity with the maximum UV dose. Full test report is provided in appendix 3 of this document.

Alga Growth Inhibition Test

To evaluate the 96-hour effects of ballast water treated by OceanDoctor BWMS to marine algae(*Chlorella spp.*), NOAEC, I_rC_{50} and I_yC_{50} values based on cell densities were calculated for both high salinity(32‰~34‰) and medium salinity(16‰~18‰) at the end of the test.

No significant toxicity effect was observed in alga (*Chlorella spp.*) growth inhibition test. The results are summarized in Table 7.2 and Table 7.3.

Table 7.2 Results of alga growth inhibition test for high salinity(32‰~34‰) ballast water treated by OceanDoctor BWMS

	Species	NOAEC	I_rC_{50} and I_yC_{50}		Reference
		Conc.(%)	Conc.(%)	Duration	
Algae	*Chlorella spp.*	>100	>100	96 h	OECD 201

Table 7.3 Results of alga growth inhibition test for medium salinity(16‰~18‰) ballast water treated by OceanDoctor BWMS

	Species	NOAEC	I_rC_{50} and I_yC_{50}		Reference
		Conc.(%)	Conc.(%)	Duration	
Algae	*Chlorella spp.*	>100	>100	96 h	OECD 201

Acute toxicity test

Fish: To evaluate the acute effects of ballast water treated by OceanDoctor BWMS to Bareneck goby(*Ctenogobius gymnauchen*), LC_{50} values based on survival numbers were calculated for both high salinity(32‰~34‰) and medium salinity(16‰~18‰) at the end of the test.

Invertebrate: To evaluate the acute effects of ballast water treated by OceanDoctor BWMS to marine invertebrate (*Neomysis awatschensis*), LC_{50} values based on survival numbers were calculated for both high salinity (32‰~34‰) and medium salinity (16‰~18‰) at the end of the test.

No significant toxicity effect was observed in fish and invertebrate acute toxicity test. The results are summarized in Table 7.4 and Table 7.5.

Table 7.4 Results of acute toxicity test for high salinity(32‰~34‰) ballast water treated by OceanDoctor BWMS

	Species	LC_{50}		Reference
		Conc.(%)	Duration	
Fish	*Ctenogobius gymnauchen*	>100	96 h	OECD 203
Invertebrate	*Neomysis awatschensis*	>100	96 h	EPA-821-R-02-012 OPPTS 850.1035

Table 7.5 Results of acute toxicity test for medium salinity(16‰~18‰) ballast water treated by OceanDoctor BWMS

	Species	LC_{50}		Reference
		Conc.(%)	Duration	
Fish	*Ctenogobius gymnauchen*	>100	96 h	OECD 203

Invertebrate	*Neomysis awatschensis*	>100	96 h	EPA-821-R-02-012 OPPTS 850.1035

Chronic toxicity test

Fish: To evaluate the chronic effects of ballast water treated by OceanDoctor BWMS to Bareneck goby (*Ctenogobius gymnauchen*), LC_{50} values based on survival numbers were calculated for both high salinity(32‰~34‰) and medium salinity(16‰~18‰) at the end of the test.

Invertebrate: To evaluate the chronic effects of ballast water treated by OceanDoctor BWMS to marine invertebrate (*Neomysis awatschensis*), LC_{50} values based on survival numbers were calculated for both high salinity (32‰~34‰) and medium salinity(16‰~18‰) at the end of the test.

No significant toxicity effect was observed in fish and invertebrate chronic toxicity test. The results are summarized in Table 7.6 and Table 7.7.

Table 7.6 Results of chronci toxicity test for high salinity (32‰~34‰) ballast water treated by OceanDoctor BWMS

	Species	LC_{50}		Reference
		Conc.(%)	Duration	
Fish	*Ctenogobius gymnauchen*	>100	7 d	EPA-821-R-02-014
Invertebrate	*Neomysis awatschensis*	>100	7 d	EPA-821-R-02-014 OPPTS 850.1350

Table 7.7 Results of chronci toxicity test for medium salinity (16‰~18‰) ballast water treated by OceanDoctor BWMS

	Species	LC_{50}		Reference
		Conc.(%)	Duration	
Fish	*Ctenogobius gymnauchen*	>100	7 d	EPA-821-R-02-014
Invertebrate	*Neomysis awatschensis*	>100	7 d	EPA-821-R-02-014 OPPTS 850.1350

Refer to appendix 3 of this document for detail.